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## COMPARATIVE SDS-PAGE ANALYSIS OF THE PROTEIN FRACTIONS OF SEMI-SPREADING GROUNDNUT CULTIVARS

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### ABSTRACT

Groundnut (*Arachis hypogaea*) ranks as the fourth most important source of global edible vegetable oil and the third for vegetable protein. At the Main Oilseed Research Station in Junagadh, ten semi-spreading groundnut varieties were studied to identify four protein fractions—albumin, prolamin, globulin, and glutelin—using SDS-PAGE profiling. The process involved centrifuging groundnut seed powder with water, sodium chloride, phosphate buffer, and alcohol. Albumin content ranged from 13.65% to 21.4%, globulin from 72.05% to 78.5%, glutelin from 1.38% to 2.69%, and prolamin from 1.47% to 4.39%. Globulin was the predominant fraction. SDS-PAGE analysis of the varieties revealed significant genetic differences, indicated by the number of bands and MW-Rf values for each protein fraction. Albumin and globulin showed the highest MW-Rf values, whereas glutelin and prolamin had minimal bands. This study concludes that the qualitative and quantitative differences in seed protein profiles are effective for distinguishing varieties, with MW-Rf values in SDS-PAGE being a promising method for varietal identification based on protein composition.

**Key words:** semi spreading, protein fraction, SDS-PAGE,

### Introduction

Groundnut is of genus *Arachis* and species of *hypogea* is a member of the legume family *Fabaceae* with chromosome number  $2n=4x=40$ . In India it is an important in term of oilseed crop and widely grown on rainfed and irrigated lands. Based on the growth habit groundnut are varieties commonly classified into three groups viz spreading semi spreading and bunch types. India is the second largest producer of groundnut in world. Around 88% of the groundnut area and production in India is concentrated in five states: Andhra Pradesh, Gujarat, Karnataka, Tamil Nadu, and Maharashtra. Nearly 83% of the total area is under rainy-season groundnut and the other 17% is cultivated during the post rainy season.

Pods containing seeds are produced below ground. Proximate composition seed of groundnut seeds contains from 23.5 to 26.6%, fat content ranged from 49.8-53.4%, Ashes of about 1-2% Campos *et al.*, (2009). Gore *et al.*, (2023) study reassurance for groundnut food processors, breeders, and geneticists in deciding varying oil % as well as changes in the ratio of oleic to linoleic acid in groundnut fully-fledged programs. Groundnut protein is increasingly becoming important as food and feed sources, especially in developing countries Ayoola *et al.*, (2012). Protein content and composition influenced by environment, season and location effects, growing conditions, stage of maturity and genotypes. Additional beneficial nutrients indigenous to groundnuts include

vitamin E, L-arginine, soluble and insoluble fiber as well as water and lipid-soluble natural phenolic antioxidant.

The first attempt to isolate the proteins of *Arachis hypogaea* L. by Ritthausen (Neucere, 1969). The groundnut seed contains 32 different proteins comprised of albumins and globulins. The seed storage proteins are mainly composed of arachin (*legumin*), conarachin (*vicilin*) - I, II fractions Moore *et al.*, (1989). These subcellular organelles, the aleurone grains, constitute the bulk of the reserve proteins that supply nitrogenous materials for protein synthesis upon germination. Isolation of these particles by Dieckert *et al.*, (1962) indicates that they represent the major source of proteins in the cotyledon. Groundnutseed composed of families of 2S, 7S, and 11S proteins that can be subdivided in homology groups. 11S proteins are more diverse than 2S and 7S proteins in groundnut seeds, but 2S and 11S subgroups are very similar in the A and B genomes of *Arachis*. Globulins have long been considered as storage proteins while, until recently, albumins have been thought of as metabolic protein [Dieckert *et al.*, (1962). Chiouet *al.*, (1990) purified arachin and conarachin from groundnut seeds by phosphate buffer and characterized on SDS-PAGE.

Protein fraction analysis in groundnut grown in Indiaspecially in Gujarat hasdearth of data or information. The present study, focuses the comparative analysis of protein fractions using SDS PAGE of four fractions, including albumins globulins, glutelin and prolamin provides new and advantageous information regarding the evolution, diversification and genomic constitution of the varieties belonging to ten semi spreading type groundnut.

## Materials and Methods

Seed material contained of ten Groundnutvarieties of semi spreading type used were provided by main oilseeds research station, Junagadh. 5g of groundnut seeds were crushed to obtain defatted powder using a Soxhlet apparatus with 250 ml of hexane, heated at 65°C for 8 hours at a rate of 150 drops per minute. Protein fractions were analyzed using the Folin-Lowry method. The defatted powder was progressively centrifuged with water, sodium chloride, phosphate buffer, and alcohol to isolate albumin, globulin, glutelin, and prolamin, respectively.

For the extraction process, 0.5 g of defatted samples were mixed with distilled water and centrifuged at 10,000 g for 6 minutes. The supernatant was used to estimate protein fractions via the Folin-Lowry method, and the loading values for PAGE analysis were calculated. The pellet from the albumin extraction was dissolved in 5 ml

of 5.0M sodium chloride solution, centrifuged for 6 minutes at 10,000 rpm, and the supernatant was analyzed for globulin using the Lowry method and SDS-PAGE, with readings taken at 660 nm. The residue from the salt extraction was re-extracted with 5 ml of 0.2 M phosphate buffer (pH 8.0) and centrifuged for 6 minutes at 10,000 rpm. The supernatant was used to estimate glutelin. Finally, the remaining pellet was treated with 5 ml of 70% alcohol and centrifuged at 10,000 rpm for 6 minutes. The supernatant was used to assay prolamin. Protein fraction assays followed the Lowry *et al.*, (1951) method.

## SDS-PAGE for seed protein profiling

All procedures and electrophoretic reagents were as described Laemmli *et al.*, (1970). Profiling of proteins was made in a 12% of separating and Staking gel (5%) with an electrophoresis unit. Protein bands were stained bromophenol blue staining solution. GEL ANALYZER (19.1) software was used for data analysis of Rf value in individual protein fraction for bands. The resulted similarity matrix was used to make dendrogram was using PAST software. GEL ANALYZER (19.1) software was used for data analysis of Rf value in individual protein fraction for bands. The resulted similarity matrix was used to make dendrogram was using PAST software.

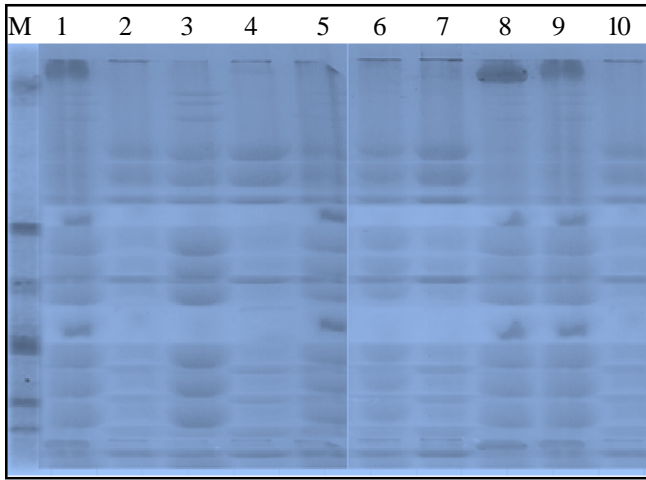
## Result and Discussion

### Protein fractions

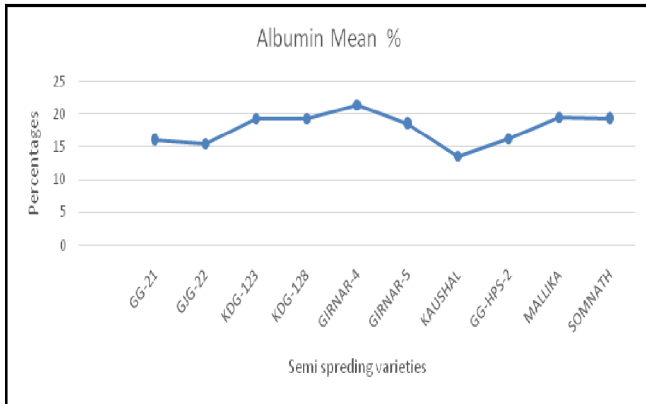
Semi spreading type varieties shown significant variation in water soluble fraction of protein among the thirty varieties (Table 1). The albumin content was found in the range of 13.65 and 21.4 % for ten varieties from which GIRNAR-4 (21.4%) indicated the highest value for the albumin % while variety KAUSHAL (13.65%) observed the lowest value for albumin content (Fig. 2

**Table 1:** Mean % of four protein fractions.

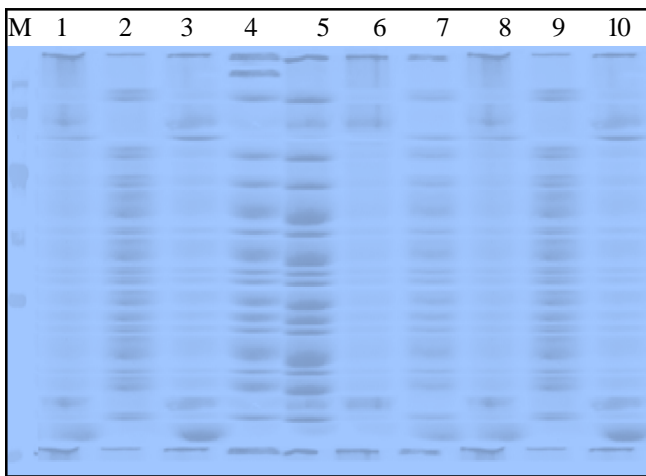
Semi spreading Genotype	Albumin Mean %	Globulin Mean %	Glutelin Mean %	Prolamin Mean %
GG-21	16.17	77.86	2.63	2.34
GJG-22	15.51	77.17	1.38	2.25
KDG-123	19.32	72.06	1.74	3.1
KDG-128	19.28	73.25	1.55	1.72
GIRNAR-4	21.4	72.05	1.8	1.47
GIRNAR-5	18.65	73.99	1.69	1.66
KAUSHAL	13.65	78.5	2.34	4.39
GG-HPS-2	16.33	75.77	2.15	2.28
MALLIKA	19.57	72.57	2.6	1.65
SOMNATH	19.41	72.98	2.69	3.17
SEm	0.3	0.7	0.13	0.144
C.D. (0.05)	0.87	2.01	0.31	0.35
C.V%	2.37	1.32	8.29	7.91



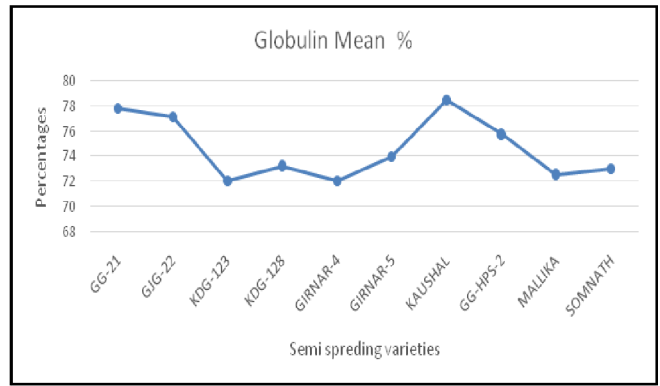
**Fig. 1:** SDS PAGE showing banding patterns for albumin fraction of semi spreading type M-standard, 11.GG-21, 12.GJG-22, 13.KDG-123, 14.KDG-128, 15. GIRNAR-4, 16. GIRNAR-5, 17. KAUSHAL, 18.GG-HPS-2, 29. MALLIKA, 20. SOMNATH



**Fig. 2:** Mean % of Albumin fractions.



**Fig. 3:** SDS PAGE electrophoresis gel showing banding patterns for globulin fraction of semi spreading type M-standard, 11.GG-21, 12.GJG-22, 13.KDG-123, 14.KDG-128, 15. GIRNAR-4, 16. GIRNAR-5, 17. KAUSHAL, 18.GG-HPS-2, 19. MALLIKA, 20. SOMNATH.

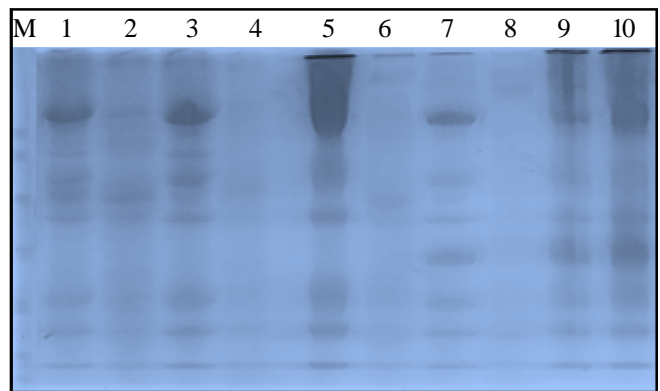


**Fig. 4:** Mean % of Globulin fractions.

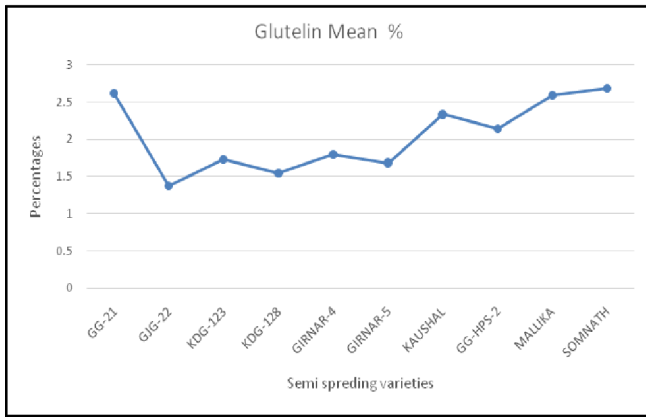
and 4). Youle and Huang (1981) showed that a substantial proportion of the total seed protein and observed albumins, comprised 20-60% of the total seed proteins. The globulin content ranged between 72.05 to 78.5 % in ten varieties. These results are in agreement with Sebei *et al.*, (2013) they found groundnut seed proteins that is globulin content in ranges between 94-95%. Glutelin content was found to be in the range of 1.38 to 2.69% (Fig. 6). This variation may be due to their solubility and differentially expression of genes for glutelin in types of varieties. The result of prolamin content in semi spreading type seed kernels was found to be significant ranged between 1.47 to 4.39 %. This variation among the varieties shown differently due to less solubility. Between the ten varieties, KAUSHAL variety was found higher percent for prolamin (4.39 %) while in GIRNAR-4 variety less value 1.47 % for prolamin reported lower value (Fig. 8).

**SDS-PAGE protein profiling analysis**

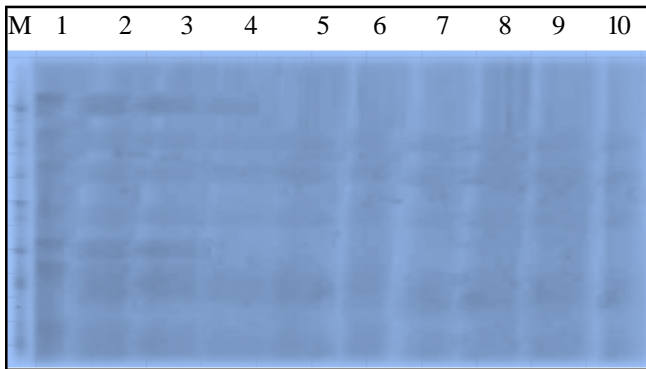
Electrophoresis was used to compare the protein composition of the different groundnuts. To identify the individual bands in this sample, BSA used as markers. The pattern of seed protein fraction of Groundnut



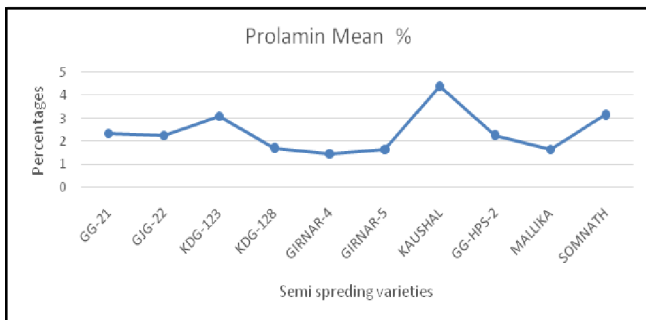
**Fig. 5:** SDS PAGE showing banding patterns for glutelin of semi spreading type M-standard 11.GG-21, 12.GJG-22, 13.KDG-123, 14.KDG-128, 15. GIRNAR-4, 16. GIRNAR-5, 17. KAUSHAL, 18.GG-HPS-2, 29. MALLIKA, 20. SOMNATH.



**Fig. 6:** Mean % of Glutelin fractions.



**Fig. 7:** SDS PAGE showing banding patterns for prolamin fraction of semi spreading type M-standard, 11.GG-21, 12.GJG-22, 13.KDG-123, 14.KDG-128, 15. GIRNAR-4, 16. GIRNAR-5, 17. KAUSHAL, 18.GG-HPS-2, 19. MALLIKA, 20. SOMNATH.

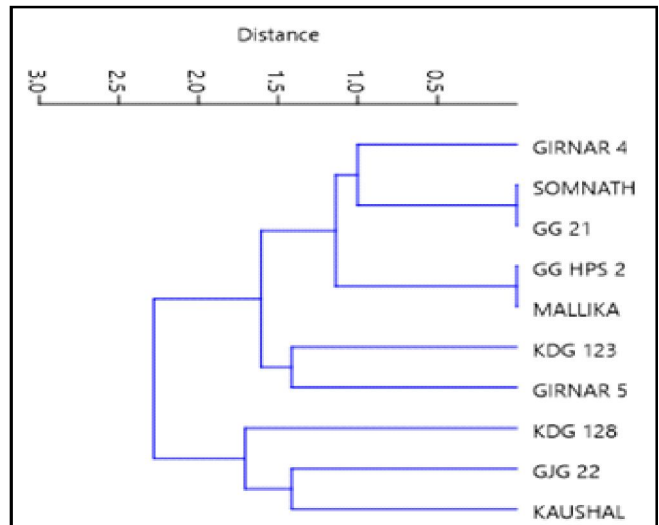


**Fig. 8:** Mean % of Prolamin fractions.

exhibited variances in banding pattern amongst the varieties on the basis of MW-Rf value. These banding pattern of SDS- PAGE had been applied for the detection of polymorphism in difference varieties of Groundnut. To explore variability among the seed kernel protein through SDS-PAGE in different type of Groundnut varieties shown the following results.

**Albumin**

SDS-PAGE profiling of albumin existing the number bands were recorded in the ten semi spreading type varieties ranged from 14 to 20. The proteins with MW-

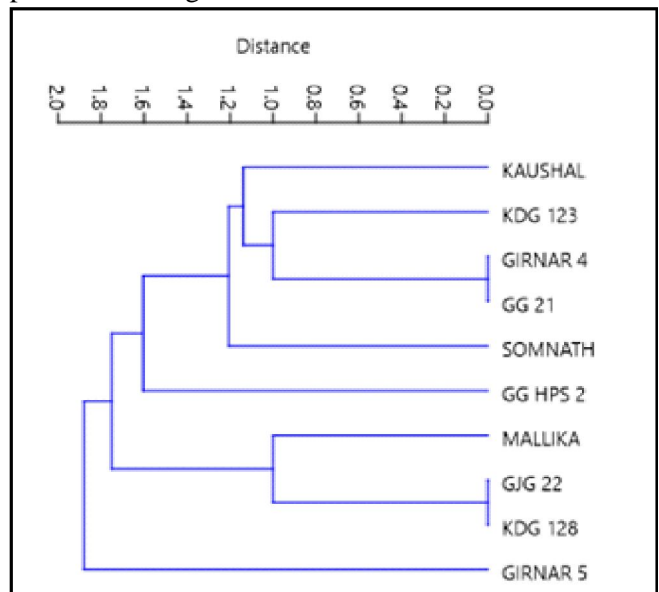


**Fig. 9:** Dendrogram generated using albumin protein of 10 Semi spreading groundnut varieties through SDS-PAGE profile.

Rf Results and discussion 77 value of 0.31, 0.351, 0.469, 0.506, 0.547, 0.991, 0.942, 0.761, 0.817, 0.906 and 0.233 were commonly seen monomorphic bands in all ten spreading varieties whereas, remaining 9 bands were polymorphic in nature (Table 2) Among the ten varieties, the maximum protein bands (20) were found to be in GG-HPS-2 and MALLIKA variety whereas minimum bands (14) were observed in KDG-128 and KAUSHAL variety of semi spreading type (Fig. 1).

**Globulin**

The outcomes of globulin fraction protein profiling comparison of variety analyzed for SDS-PAGE as presented in Fig. 3. The number bands were noticed in



**Fig. 10:** Dendrogram generated using Globulin protein of 10 Semi spreading groundnut varieties through SDS-PAGE profile.

**Table 2:** MW-Rf value on different albumin protein fraction bands of semi spreading type groundnut varieties based on SDS-PAGE.

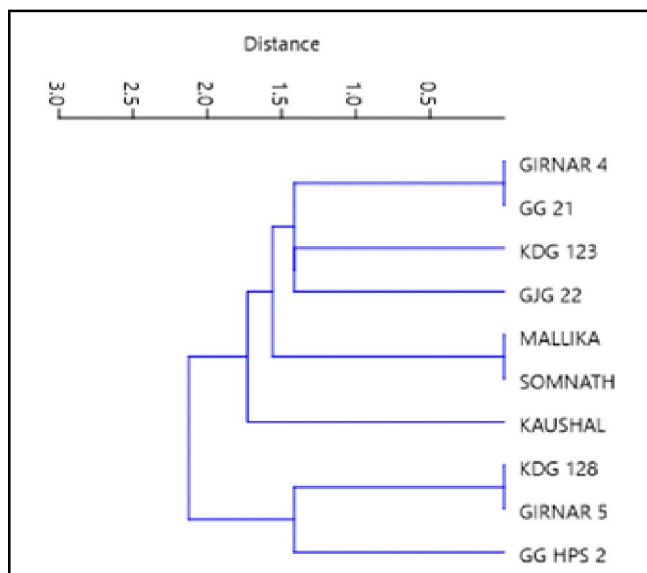
Band No.	MW-Rf	GG 21	GJG 22	KDG 123	KDG 128	GIRNAR 4	GIRNAR 5	KAUSHAL	GG HPS 2	MALLIKA	SOMNATH
1	0.012	1	1	1	1	0	1	1	1	1	1
2	0.047	0	0	0	0	0	0	0	1	1	0
3	0.102	1	0	1	0	1	1	0	1	1	1
4	0.125	1	0	1	0	1	1	0	1	1	1
5	0.161	1	0	1	1	1	1	0	1	1	1
6	0.233	1	1	1	1	1	1	1	1	1	1
7	0.31	1	1	1	1	1	1	1	1	1	1
8	0.351	1	1	1	1	1	1	1	1	1	1
9	0.404	1	1	0	0	1	1	0	1	1	1
10	0.469	1	1	1	1	1	1	1	1	1	1
11	0.506	1	1	1	1	1	1	1	1	1	1
12	0.547	1	0	1	1	1	1	1	1	1	1
13	0.6	1	0	0	0	1	0	0	1	1	1
14	0.67	1	1	1	0	1	1	1	1	1	1
15	0.723	1	1	1	1	1	0	1	1	1	1
16	0.761	1	1	1	1	1	1	1	1	1	1
17	0.817	1	1	1	1	1	1	1	1	1	1
18	0.906	1	1	1	1	1	1	1	1	1	1
19	0.942	1	1	1	1	1	1	1	1	1	1
20	0.991	1	1	1	1	1	1	1	1	1	1

the ten semi spreading type varieties ranged from 17 to 20. Ten varieties were analyzed through SDS-PAGE it found to be clear that a great variation could be observed in banding pattern and MW-Rf value of groundnut semi spreading varieties. Highest number of bands (20) and lowest (17) bands were recorded in the variety GG-21 and GIRNAR-4 and GJG-22, KDG-128 and GIRNAR-5, respectively. Total eleven monomorphic band were found with band Rf viz., 0.022, 0.217, 0.416, 0.460, 0.558,

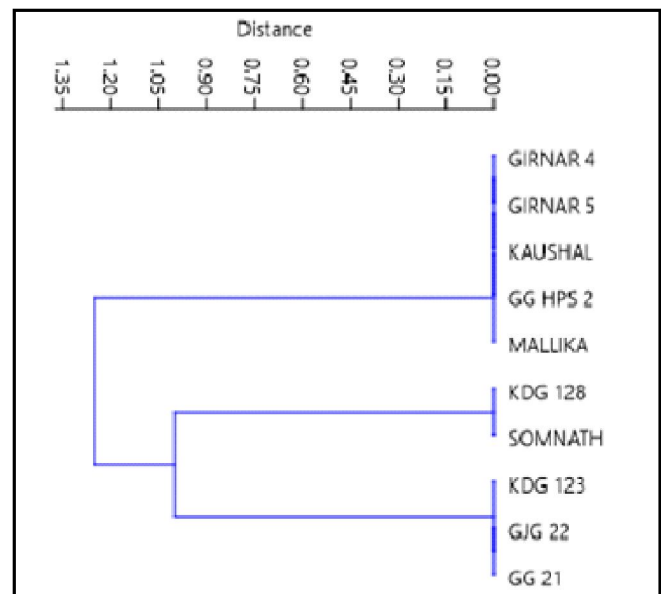
0.595, 0.632, 0.705, 0.763, 0.831 and 0.875 whereas 9 polymorphic bands were detected in SDS PAGE viz., 0.068, 0.096, 0.125, 0.265, 0.333, 0.518, 0.803 and 0.978 (Table 3).

### Glutelin

The SDS-PAGE analysis comparison of ten semi spreading varieties and the results of glutelin fraction protein profiling as shows in Fig. 5. The number of bands



**Fig. 11:** Dendrogram generated using Prolamin protein of 10 Semi spreading groundnut varieties through SDS-PAGE profile.



**Fig. 12:** Dendrogram generated using Glutelin protein of 10 Semi spreading groundnut varieties through SDS-PAGE profile.





in ten bunch type groundnuts in glutelin fraction found to be ranged from 5 to 10. The bands with MW-Rf value of 0.374, 0.489, 0.845 and 0.937 were present in all (monomorphic) the semi spreading whereas remaining 6 were polymorphic viz., MW-Rf value of 0.052, 0.193, 0.257, 0.321, 0.593 and 0.762. The maximum glutelin Results and discussion 87 protein fraction bands (10) were found to be in MALLIKA and SOMNATH variety whereas minimum bands (5) were observed in GG-HPS-2, KDG-128 and GIRNAR-5 varieties of semi spreading type (Table 4).

### Prolamin

Comparison of ten semi spreading variety analyzed for SDS-PAGE and the results of prolamin fraction protein profiling as presented in Fig. 7. In the ten semi spreading type varieties total 6 bands were found in SDS-PAGE analysis for prolamin protein fraction. The monomorphic bands with MW-Rf value of 0.381, 0.522, 0.625, 0.741, and 0.911 were present in all semi spreading. Whereas remain two were polymorphic in nature. Among the varieties, the maximum protein bands (6) were found to be in GG-21, GJG-22, KDG-123 and SOMNATH variety whereas minimum bands (4) were observed in GIRNAR-4, KAUSHAL GG-HPS-2, MALLIKA and GIRNAR-5 varieties of semi spreading type (Table 5).

### Discussion

In their analysis of 50 groundnut (*Arachis hypogaea* L.) accessions for albumin in SDS-PAGE, Singh *et al.*, (2018) reported similar observations. 15-21 bands were discernible in the SDS-PAGE examination of the albumin seed protein fraction also they found similar results for protein bands in groundnut seeds. Their findings demonstrated the resolution of 22 protein bands by the globulin protein fraction. Senakoon *et al.*, (2015) studied the seed prolamins derived by alcohol extraction and then acetone precipitation, and they found similar results for protein bands in groundnut seed. The profile included polypeptides ranging in size from tiny to about 66 kDa. At 22.4 kDa to below 14.4 kDa, four main bands were distinct. The possible identification of protein fractions that have not yet been characterized might lead to the investigation of novel functional traits, which would have an influence on a number of industries, including food science, plant biology, and nutrition. To properly comprehend the ramifications of these discoveries, more research including protein identification and characterization is needed. Albumin% and globulin% content found to be in range of 16.2 to 20.43% and 72.05-78.5% for the spreading type of groundnut seeds Gore *et al.*, (2022). The presence or absence of peptide bands can be useful for identification of landrace among others

Saminu *et al.*, (2021). This study sets the path for a thorough comprehension of groundnut proteins and potentially major improvements in groundnut breeding, allergy reduction, and uses in the food business. Our findings further demonstrate the effectiveness of SDS-PAGE as a straightforward yet reliable technique for protein analysis, encouraging its expanded use in research on other leguminous and non-leguminous crops.

### Conclusion

Researchers engaged in the analysis of groundnut proteomes can leverage the findings of experiments to enhance their scientific endeavors. This investigation offers valuable insights into the groundnut genotypes, particularly in identifying high-quality features through the examination of protein profiles across different types. The utilization of SDS-PAGE analysis in our study significantly advances our comprehension of groundnut protein fractions. This technique enabled us to effectively isolate and characterize various protein fractions, providing crucial insights into the intricate and diverse nature of proteins present in groundnuts. Given that these proteins may play a role in both the nutritional and allergic aspects of groundnuts, our results underscore the potential benefits of conducting a more comprehensive study on protein fractions.

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